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Effect of the Variation of Loading Frequency on Surface Failure of Bovine Articular Cartilage

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Summary

Background: Mechanical loading of synovial joints can damage the articular cartilage surface and may lead to osteoarthritis. It is unknown if, independent of load, frequency alone can cause failure in cartilage. This study investigated the variation of articular cartilage surface damage under frequencies associated with normal, above normal and traumatic loading frequencies.

Method: Cartilage on bone, obtained from bovine shoulder joints, was tested. Damage was created on the cartilage surface through an indenter being sinusoidally loaded against it at loading frequencies of 1, 10 and 100 Hz (i.e. relevant to normal, above normal and up to rapid heel-strike rise times, respectively). The frequencies were applied with a maximum load in the range 60-160 N. Surface cracks were marked with India ink, photographed and their length measured using image analysis software.

Results: Surface damage increased significantly ($p<0.0001$) with frequency throughout all load ranges investigated. The dependence of crack length, *c,* on frequency, *f*, could be represented by, $c = A(log_{10}(f))^2 + B(log_{10}(f)) + D$ where A = 0.006 ± 0.23, B = 0.62 ± 0.23 and D = 0.38 ± 0.51 mm (mean ± standard deviation).

Conclusion: The increase in crack length with loading frequency indicated that, increased loading frequency can result in cartilage becoming damaged. The results of this study have implications in the early stages of osteoarthritis.

Keywords: Articular cartilage, Crack, Failure, Frequency, Mechanical loading, Osteoarthritis.

1. Introduction

In this study, experimental damage of the articular cartilage surface was produced by applying five sinusoidally varying compressive force ranges over three magnitudes of loading frequency. Articular cartilage can become damaged when subjected to repetitive mechanical loading[1–3]. However, the mechanism by which the surface of cartilage becomes damaged under loading frequencies associated with normal, above normal and rapid heel-strike rise time is unknown.

Articular cartilage is a compliant layer covering the much stiffer bone ends of a joint, preventing high contact stresses which could ultimately damage the bone in a joint. Cartilage needs, therefore, to be able to deform in order to increase the total surface area for contact, thereby reducing the overall stress[2,4]. Cartilage also provides smooth bearing surfaces, with surface roughness of 80-170 nm, in freely moving synovial joints[5].

The most recognized feature of osteoarthritis is the progressive damage of articular cartilage, resulting in impaired joint motion, severe pain, and disability[6]. The articular cartilage surface begins to change from a smooth to a rough or fibrillated appearance in early osteoarthritis[7]. Once damaged, it has a very limited ability to repair itself[8,9].

Rapid heel strike rise times during gait have been associated with the early onset of osteoarthritis[10,11]. Heel strike rise times in the normal population have been previously determined to be typically 100-150 ms. However, a subset of the population with heel-strike rise times from 5 to 25 ms have been identified and are fast enough to create impulsive loadings[12]. Repetitive impulsive/traumatic loading was found to provoke osteoarthritis in

animal experiments[11]. In general, heel-strike rise times of 500 ms corresponds to a loading frequency of 1 Hz, whereas a rapid heel-strike rise time such as 5.4 ms is equivalent to a loading frequency of 92 Hz[12]. Cyclic compressive loading has been used to subject the surface of cartilage to damage[1,2]. However, little is known about the role of loading frequency in the initiation and progression of damage in cartilage.

Previous studies[12–14] which investigated changes in cartilage viscoelastic properties with frequency suggested that the likelihood of cartilage failure increases with loading frequency. This was suggested, because at higher frequencies the ability of the tissue to store energy, for elastic recoil, increased. It was suggested that if the energy available for storage exceeded a certain level it might induce damage to the cartilage. Damage caused by increasing the loading frequency has been suggested to be different to the damage caused by increasing load only following comparisons between failure patterns from static loading tests[15,16].

Another factor associated with the failure of articular cartilage is the mechanical overload of the joint[17]. Cartilage fissures have typically been formed in the regions exposed to high loads in the joint[18,19]. Therefore, the aim of this study was to determine the variation of articular cartilage surface damage with frequencies relevant to normal, above normal and rapid heel-strike loading, and how this relationship is altered by the maximum stresses which are induced in cartilage.

2. Method

Specimen preparation

Fresh bovine shoulders, from animals less than 30 months old, were obtained from a supplier (Johnston's Butcher, King's Heath, Birmingham, UK). On arrival in the laboratory they were wrapped in tissue paper and soaked in Ringer's solution. The bovine shoulders were then sealed in plastic bags, stored in a freezer at -40°C until they were required for testing[2,12–14]. Freeze-thaw treatment has not been found to alter the mechanical properties of articular cartilage[20–22] or bone[23]**.** Prior to testing, the specimens were thawed at room temperature for approximately 2 hours[12,13]. Two 50 × 50 mm cartilage-on-bone specimens were obtained from the humeral head of a shoulder joint. Typically specimens had a total thickness of 20 mm (cartilage and subchondral bone) to allow sufficient bone for secure fixation in the test rig[12,14].The specimens were secured into a custom-made test rig using acrylic cement (WHW plastics, Hull, UK). Each specimen was then bathed in Ringer's solution at room temperature throughout the whole test, as in previous studies[2,12].

India ink (Loxley Art Materials, Sheffield, UK) was used to confirm that each cartilage surface was initially free from defects. Large scale damage was not observed on the surface of any specimen before mechanical testing; this was expected because joints were not osteoarthritic[13,24].

Mechanical testing

An experimental protocol was developed to investigate the role of frequency of loading on damage to the articular cartilage surface. Damage was created using a sinusoidally varying compressive load with a solid cylindrical indenter. The indenter had a 5.2 mm diameter flat

circular face with a 0.5 mm radius bevel in order to avoid high stresses around its edge. A Bose ElectroForce ELF3200 material testing machine, operated under the control of WinTest software (Bose Corporation, ElectroForce systems Group, Minnesota, USA) was used to perform indentation on the articular cartilage specimens. A total of 40 specimens were obtained from 20 bovine shoulder joints; it is unknown if any of the joints were from the same animal, so in the calculations of the 95% confidence intervals it was assumed that the joints came from 10 animals to avoid any possibility of dependant observations. Three sites, free from pre-existing lesions, were chosen for testing on each tissue sample over three magnitudes of loading frequency (i.e. one frequency per test site). Therefore, a total of 120 distinct test sites have been analysed for crack measurements. Frequencies tested ranged from normal, above healthy-gait and corresponding to rapid heel-strike rise times, using 1 Hz, 10 Hz and 100 Hz respectively[12, 13]. Each test consisted of the cartilage tissue samples being loaded for 10,000 cycles[3] for each loading frequency individually. The number of cycles was kept constant for all tests over all three magnitudes of frequency, in order to be able to observe the change in damage when only frequency was altered. Surface effects may extend up to 1 mm from the loaded site in healthy cartilage[25]; therefore, an average distance of 5 mm was kept between the test sites. Five sinusoidally varying compressive force ranges were used for testing: 6-60 N, 9-90 N, 10-100 N, 12-120 N and 16-160 N. The maximum applied loads induced a nominal compressive stress of 2.8 MPa, 4.2 MPa, 4.7 MPa, 5.6 MPa and 7.5 MPa, respectively. Eight samples were tested for each loading range at 1 Hz, 10 Hz and 100 Hz. The loading ranges were chosen to determine in which loading range and frequency the surface damage was initiated and how it changes with load or frequency. These values were chosen based on preliminary tests and the stresses induced by the maximum load.

The thickness of each site tested was measured after testing, using an established technique which has been described previously[12,13,26]. Briefly, a sharp needle was pushed through the cartilage layer up to the underlying bone using the testing machine described above. The thickness of the cartilage was determined from the difference in displacement readings at two points where the needle comes into contact with the cartilage surface and the point at which the needle contacts the cartilage/bone interface.

Analysis of cartilage surface damage

India ink was applied to the cartilage surface to highlight any signs of failure following each test[1,24]. The cartilage surface was then photographed using a DSC-R1 Cyber-shot© digital camera (10MP, 5 x Optical Zoom) 2.0" (Sony Corporation, 6-7-35 Kitashinagawa, Shinagawa-ku, Tokyo, Japan) after each test. A scale-bar was included in each image, positioned in the field of view. Digital images were analysed using ImageJ (version 1.48, Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA). Surface damage was observed on the surface of cartilage as cracks and fissures. Lines were drawn manually along the length of all the cracks and fissures. The software was used to calculate their total length (in mm) with a 0.1 mm precision. The length of cracks and fissures were added together for each test site[2]. Image analysis measurements were repeated twice by one individual to ensure the measurements were repeatable.

Data analysis

The mean total crack length against the three frequencies (1 Hz, 10 Hz and 100 Hz) was analysed for all load ranges using Sigmaplot Version 11.0 (Systat Software Inc., London, UK).

95% confidence intervals were calculated with the n values shown in Figure 1, so that independent observations could be assumed[27]. The relationship between total crack lengths against maximum loads was analysed in order to determine the variation of crack length when the maximum load range was altered. A polynomial or linear regression was used to fit a curve or line to the data. A p value < 0.05 indicates that the curve or line fit was significant.

3. Results

A sample image taken from five cartilage samples at the five loading ranges is shown in Figure 2. At lower loading frequencies such as 1 Hz and 10 Hz, cracks and fissures on the surface of articular cartilage following cyclic tests appeared to be single or parallel lines. However, at 100 Hz, the loaded region contained a greater number of branches. Cracks and fissures also increased in length with frequency for all samples, except for the lowest loading range of 6-60 N where there were no cracks at 1 or 10 Hz. There also appeared to be more crack branches at higher frequencies from qualitative observations (Figure 2).

The crack length was found to increase with loading frequency. This was true for individual tissue samples (Figure 3) and also when the mean values from 8 tissues samples were used (Figure 4). The mean cartilage thickness of specimens was 2.14 mm (range: 1.8–3.6 mm). Figure 4, shows the mean total crack length against frequency of 1, 10 and 100 Hz over five load ranges. The first signs of failure were observed in the loading range of 6-60 N (maximum peak nominal stress of 2.8 MPa) at 100 Hz. Maximum damage was observed in the loading range of 16-160 N (maximum peak nominal stress of 7.5 MPa) at 100 Hz. Signs of damage at a loading frequency of 1 Hz and 10 Hz were first observed in 10-100 N (maximum

peak nominal stress of 4.7 MPa) and 9-90 N (maximum peak nominal stress of 4.23 MPa), respectively.

The crack length increased significantly ($P<0.05$) with increasing frequency for all loading ranges. Second order polynomial curves (equation 1) were found to fit the data well for all sites tested in the form:

$$c = A(log_{10}(f))^2 + B(log_{10}(f)) + D \qquad \text{for} \quad f \geq 1 \qquad (1)$$

where *c* is the mean total crack length, *f* represents frequency and A, B and D are constants. (Table I).

The crack length increased with increasing the maximum load (Figure 5). Mean total crack length increased by 1.1, 2.4 and 5.1 mm at loading frequencies of 1, 10 and 100 Hz, respectively. A linear relationship was found to fit the experimental data in the form:

$$c = EL + G \qquad \text{for} \quad 60 \leq L \leq 160 \qquad (2)$$

where *c* is the mean total crack length, *L* is the maximum load and *E* and *G* are constants. Mean values of the constants *E* and *G* were 0.02 $mm.N^{-1}$ (SD 0.019, range 0.013–0.051 $mm.N^{-1}$) and -1.74 mm (SD 0.77, range -2.54 – -1 mm), respectively.

4. Discussion

The results from this study show that surface cracks can form purely by mechanical means, and this progression is frequency dependant. This study has investigated the dependency of surface damage on loading frequency. Frequencies of 1, 10 and 100 Hz were used as they correspond to normal gait, above healthy gait heel-strikes and similar to traumatic heel-strike rates, respectively[1-14,20,28]. The results show that repetitive mechanical loading of

articular cartilage causes damage in the form of crack and fissures to its surface. Crack length was found to increase with increasing loading frequency; this increase was also found to be greater at higher maximum loads. An increase in cartilage damage with increasing the loading frequency was predicted in previous studies[12,14,28]. This prediction was made because of the change in viscoelastic properties of articular cartilage with frequency. At frequencies of up to 92 Hz, there was an increase in storage to loss modulus, i.e. the ability of the material to store energy rather than dissipate it increased[12]. The main mechanism for releasing the excess energy was suggested to be by the formation of cracks. The proposed mechanism was consistent with increased cartilage failure which occurs with increasing energy during impact loading *in vitro*[29,30].

Increase in the length of cracks and fissures during mechanical cyclic loading have been reported in previous studies, but only up to frequencies of 0.1 Hz [1] or 0.5 Hz [2] and 1 Hz [31]. In the current study damage was created on cartilage samples by applying 10,000 cycles over five load ranges with peak stresses from 2.8 to 7.5 MPa. The change in damage was observed when frequency alone was altered. Ewers *et al*. (2001)[32] who investigated the propagation of damage in rabbit cartilage after blunt impact *in vivo* also reported that the total length of fissures increased during the first 4 months following impact tests.

Articular cartilage is subjected to various ranges of peak stresses under light to moderate activities which typically range from 1 to 6 MPa[33,34]. Peak stress amplitudes, under more vigorous activities in natural joints have been estimated up to 12 MPa[35] or even 18 MPa[36]. The maximum peak stresses used in this study were from 2.8 to 7.5 MPa, which is similar to peak stresses acting on cartilage during light to moderate activities.

During the normal gait cycle for walking there is a rapid rise in foot-to floor reaction force from zero to a peak load of over body weight during the first 100 ms after heel strike[37,38]. These forces results in the deformation of articular cartilage. Thus, ground reaction forces at heel-strikes provide indirect information about internal joint loading. This is because a peak ground reaction force corresponds with the timing of peak loads in joints[38-41]. Therefore the increase in the loading rate of the ground reaction force (shorter rise times) will be in correlation with the changes in rise times of the reaction forces acting on articular cartilage in lower limb joints of the body[37].

It has been suggested that impulsive/traumatic rise times during heel strike could be implicated in the onset of osteoarthritis[11]. Repetitive mechanical loading can create fissures in the surface of cartilage[1]. Significant increase in crack length with frequency in peak stresses which resembles peak stresses acting on cartilage during light and moderate activities shows the important role of loading frequency in the initiation and propagation of damage on the surface of articular cartilage in this study. Other studies[12,13,28] have reported that the ability of cartilage to store and dissipate energy is altered with loading frequency. This may change the stress transfer to the underlying bone which leads to bone stiffening and ultimately failure of articular cartilage[13]. However, energy transfer between cartilage and subchondral bone has not been investigated in this study.

The effect of increasing the loading frequency was investigated in a comparable study[42] when shear force was used to create fractures on the surface of articular cartilage experimentally. Results from this study showed that with increasing the speed of the

applied load (increasing the frequency), surface cracks appeared on the surface of cartilage whereas low speed loads with the same energy, did not create cracks on the surface of cartilage.

The effect of slowly applied loads and suddenly applied loads on articular cartilage was suggested to differ considerably in previous studies[42-45]. This was associated with the deformation of cartilage under different loading frequencies with displacement of water inside and outside of the collagen network. These studies assumed cartilage as a biphasic material composed of a solid matrix phase (20% of the total mass by weight) and an interstitial fluid phase (80%)[44]. It is not clear why the cartilage surface shows signs of cracking following cyclic tests, a behaviour mostly seen in brittle materials[46,47]. However, using a theoretical model[48], it has been shown that increasing the loading frequency, also increases the flow of interstitial fluid inside the cartilage tissue. This fluid flow in and out of the collagen matrix is eventually inhibited together at higher frequencies; therefore cartilage acts as a brittle, largely incompressible elastic solid. The biphasic mechanism was explained by assuming the solid matrix, to be a porous, permeable elastic solid and the interstitial fluid to be movable within the tissue[49]. Both phases were modelled to be intrinsically incompressible and the diffusive drag forces between the two phases resulted in the viscoelastic behaviour of the tissue[50,51]. However, this mechanism was not supported, when viscoelastic properties of cartilage were measured up to 10 Hz[14] or 90 Hz[12] as almost no variation was observed in loss modulus/stiffness with increasing the loading frequency. This might be due to the constraining effect of the underlying bone[14,52]. The underlying bone has previously been predicted to prevent an increase in loss modulus[53].

Fluid flow is not the only possible mechanism which is involved in cartilage deformation and ultimately failure. Another alternative is to treat cartilage as a hydrated gel[54,55], undergoing glassy transition (transition from soft to glassy material), under high loading frequencies. This has been discussed in several studies[12,56,57] because of the existence of hydrated proteoglycans in the cartilage matrix which act as a natural hydrogel under deformation. This is consistent with increased injury observed with increased stiffness under increased loading frequencies[42].

Articular cartilage is a composite material in which collagen fibrils provide tensile reinforcement. The alignment of collagen is different in every layer of cartilage[52,56,58]. One of the mechanisms which has been suggested as being involved in crack growth under repetitive loading on the cartilage surface is the tension developed in the superficial layer of the cartilage surface (10-20 % of cartilage thickness)[59]. The investigation of failure propagation through the depth of articular cartilage will be the subject of a future study.

5. Conclusion

It can be concluded from this study that surface damage to cartilage following sinusoidal loading increased with frequency throughout all load ranges investigated. Cracks appeared on the surface of articular cartilage in lower load ranges (below a maximum peak stress of 4.7 MPa) only at loading frequencies which are associated with impulsive/traumatic and above healthy gait heel-strike rise times, 100 Hz and 10 Hz, respectively. However, cracks appeared at all loading frequencies in higher maximum load ranges (above maximum peak stress of 4.7 MPa). Variation of damage with different loading frequencies has implications in the early stages of osteoarthritis.

6. Acknowledgements

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7. Contributions

HS carried out the experimental work, study design, data analysis and drafting the manuscript. DETS and DME participated in the study design, data analysis and revising the manuscript. All authors have read and approved the final manuscript.

8. Role of the funding source

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9. Competing interests

The authors declare they have no competing interests.

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Figure legends

Figure 1 –Number of independent observations (n) used for calculating the 95% confidence intervals in Figures 4 and 5. Each load range was tested at three frequencies on distinct test sites (120 tests).

Figure 2 –Representative images taken from cartilage samples following cyclic loading. The black circles show the loaded region. Loading ranges included were (a) 6-60 N, (b) 9-90 N, (c) 10-100 N, (d) 12-120 N and (e) 16-160 N. The formation and increase in crack length can be generally observed with increasing load and frequency. India ink was applied to the cartilage surface to easily distinguish the surface cracks. A scale bar is included in every image in order to measure the surface features.

Figure 3 – Total crack length plotted against the logarithm (with base 10) of the loading frequency for testing on individual tissue samples. The three represent the load ranges of 6-60 N (●), 9-90 N (○), 10-100 N (▼), 12-120 N (△) and 16-160 N (■).

Figure 4 –Mean total crack length plotted against the logarithm (with base 10) of the loading frequency for the load ranges of 6-60 N (●), 9-90 N (○), 10-100 N (▼), 12-120 N (△) and 16-160 N (■). Second order polynomials (see equation 1) fitted the data well. Error bars represent 95% confidence intervals, for clarity only positive error bars have been included. When error bars are not visible they are smaller than the symbols used to represent the data point.

Figure 5 –Mean total crack length plotted against the maximum load for loading frequencies of 1 Hz (●), 10 Hz (○) and 100 Hz (▼).Each point in this graph represents the mean crack length of 8 measurements. The crack length is described by linear curve fits (see equation 2). *P*-value for all lines were $P<0.05$ which indicates that lines are statistically significant. R^2 is the squared correlation coefficients and shows how well the lines fit the data point. R^2 value for loading frequencies of 1, 10 and 100 Hz are 0.87, 0.97 and 0.99, respectively. Uncertainty of estimates is presented in the form of 95% confidence intervals for clarity only positive error bars have been included. When error bars are not visible it is because they are smaller than the symbols used to represent the data point.

Figures and Tables

Table I

Details of the constants from the mean total crack length against frequency curve fits.

Load range (N)	Total crack length (*c*) curve fit				
	A	**B**	**D**	**R^2**	***P*-Value**
6-60	-0.32	0.32	0	1	0
9-90	0.03	0.51	0	1	0
10-100	0.12	0.56	0.1	1	0
12-120	-0.1	0.75	0.63	1	0
16-160	0.3	0.99	1.18	1	0

The total crack length is described by lines of the quadratic form for each load range. They are characterized by constants A, B and D. R^2 is the squared correlation coefficient and shows how well the line fits the data points. If $P<0.05$ it indicates that the lines are statistically significant.

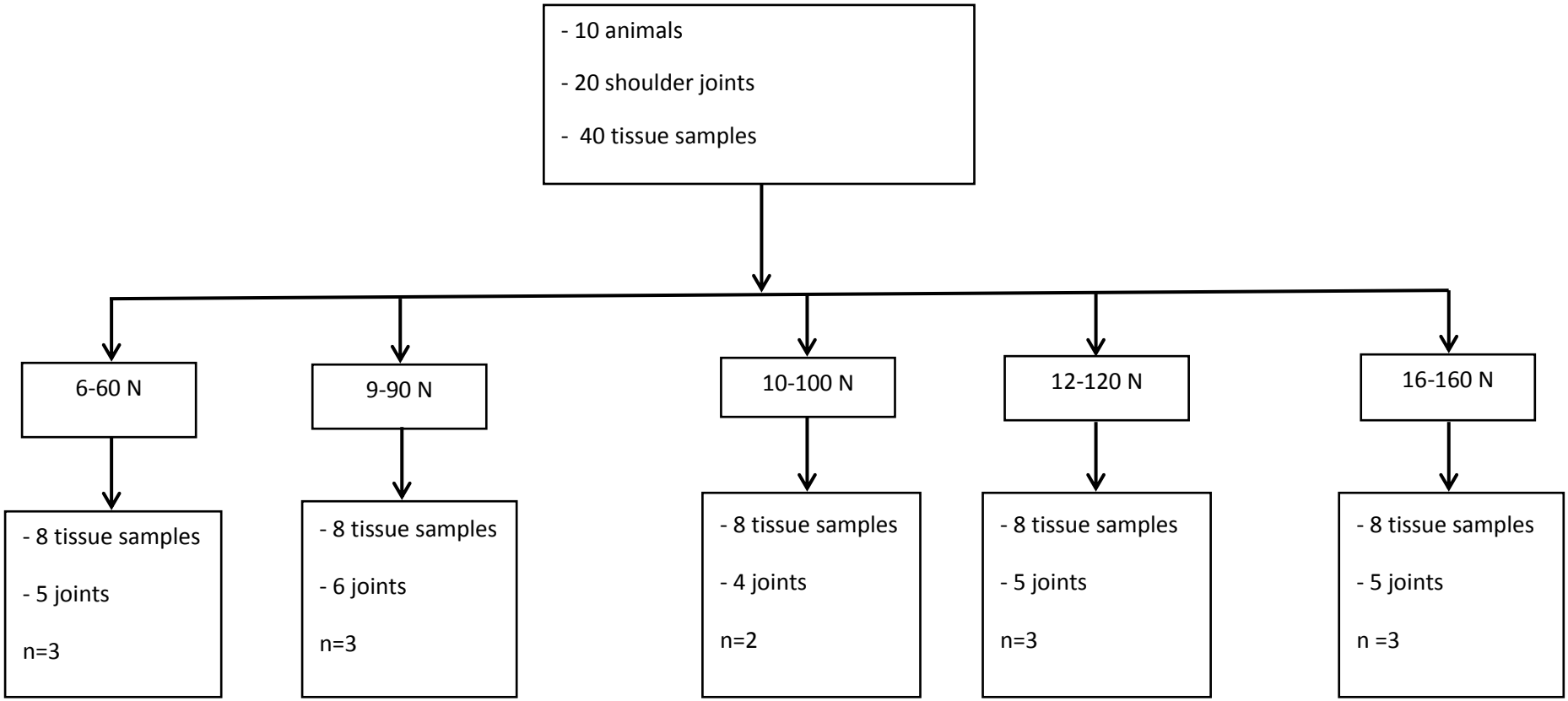
- 10 animals
- 20 shoulder joints
- 40 tissue samples
6-60 N
9-90 N
10-100 N
12-120 N
16-160 N
- 8 tissue samples
- 5 joints
n=3
- 8 tissue samples
- 6 joints
n=3
- 8 tissue samples
- 4 joints
n=2
- 8 tissue samples
- 5 joints
n=3
- 8 tissue samples
- 5 joints
n =3

Figure 1

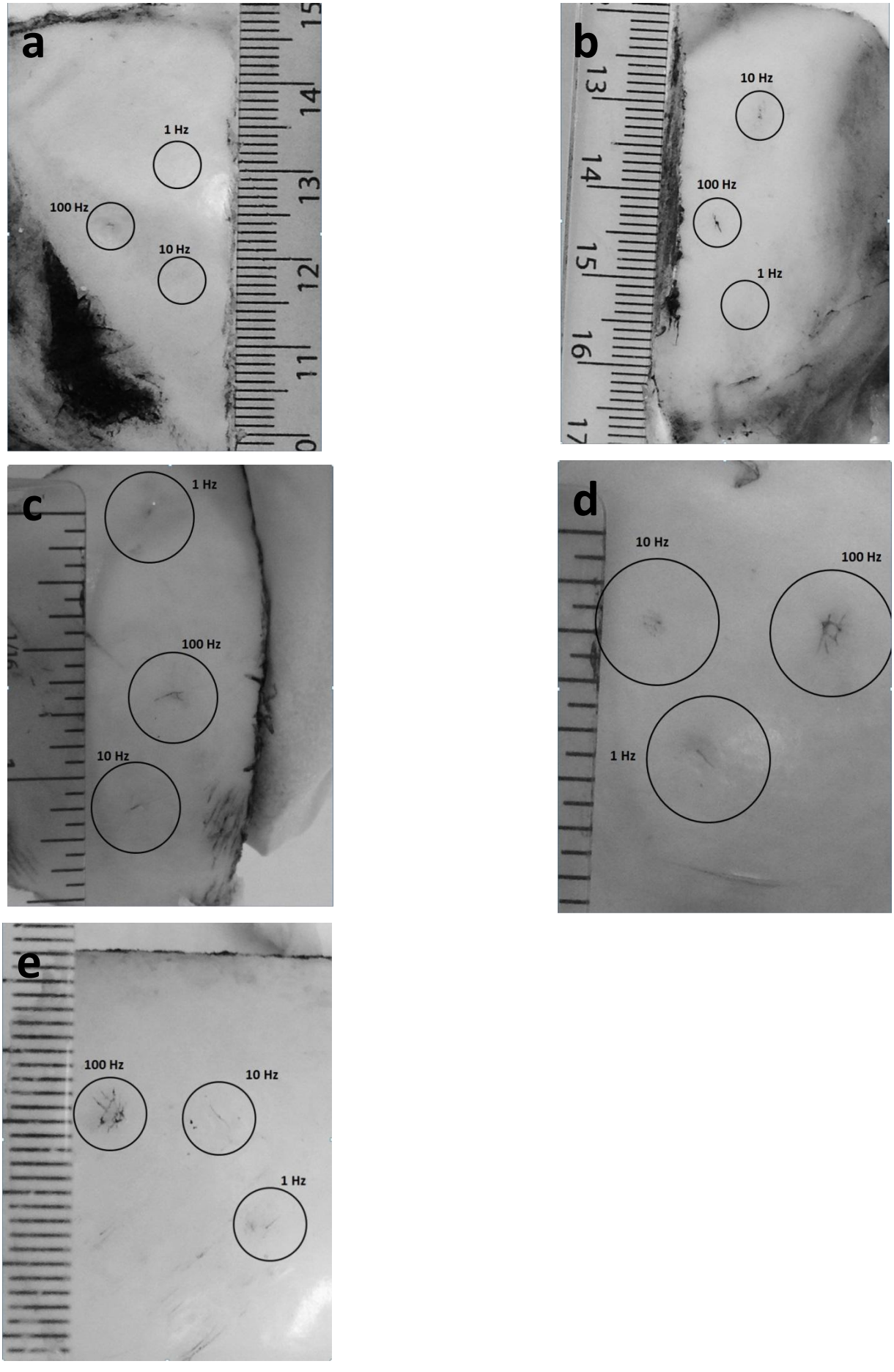
a
1 Hz
100 Hz
10 Hz
b
10 Hz
100 Hz
1 Hz
c
1 Hz
100 Hz
10 Hz
d
10 Hz
100 Hz
1 Hz
e
100 Hz
10 Hz
1 Hz

Figure 2

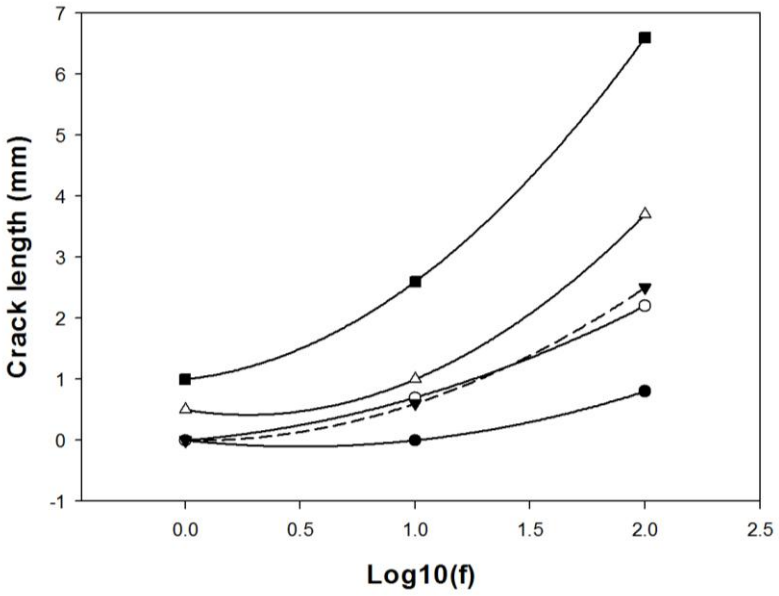

Figure 3

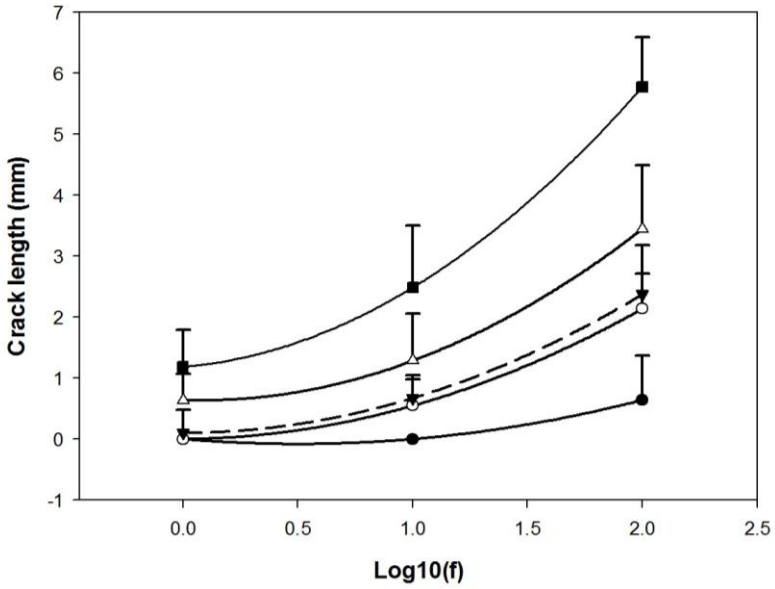

Figure 4

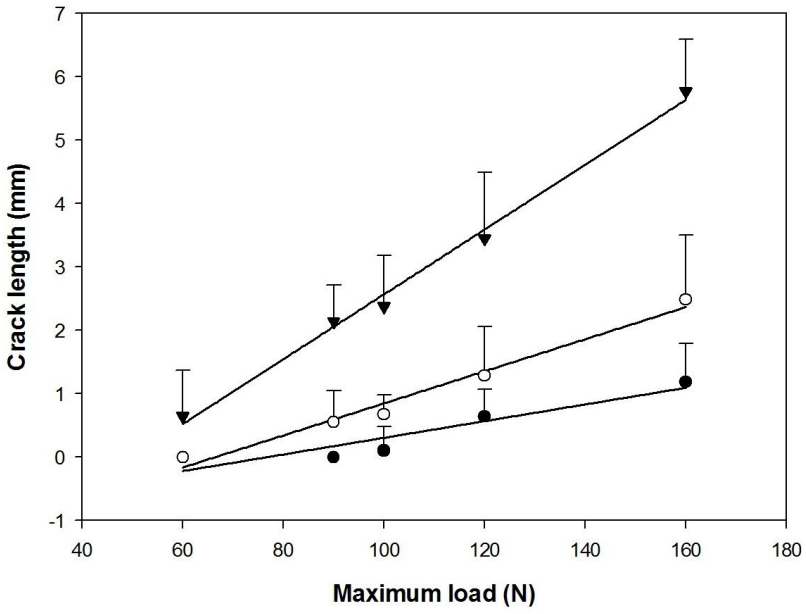
Crack length (mm)
7
6
5
4
3
2
1
0
-1
40
60
80
100
120
140
160
180
Maximum load (N)

Figure 5